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PYRIDINE NUCLEOTIDE OXIDASES AND TRANSHYDROGENASE IN ACCLIMATIZATION TO HIGH ALTITUDE

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FOREWORD

This report was prepared in the Institute of Andean Biology and the Department of Pathological Physiology, Faculty of Medicine, Lima, Peru, by:

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ABSTRACT

Activity of pyridine nucleotide oxidases and transhydrogenase has been examined in heart, liver, and rectus femoris muscle of guinea pigs native of sea level and high altitude. There was an enhanced, reduced form of diphosphopyridine nucleotide oxidase (DPNH-oxidase) and transhydrogenase activity in heart and muscle from animals adapted to high altitude. The higher activity in muscle at altitude was due solely to increase in ratio of red to white portions. Both groups showed the pigmented portion twice as active as the white one. In liver, neither the DPNH-oxidase system nor the transhydrogenase was significantly changed in their activity on a fresh-weight basis. Nevertheless, the DPNH-oxidase is higher at altitude when the activity is expressed per gram of nitrogen. The reduced form of triphosphopyridine nucleotide oxidase activity was not appreciably changed in any of the tissues. It was concluded that adaptation to high altitude is associated with apparent changes in the magnitude of the electron transport pathway. Increased activity in skeletal muscle is probably related to the tissue pigment content.

This technical documentary report has been reviewed and is approved.

Robert B Payne

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PYRIDINE NUCLEOTIDE OXIDASES AND TRANSHYDROGENASE IN ACCLIMATIZATION TO HIGH ALTITUDE

1. INTRODUCTION

Man living at high altitudes can perform muscular work with the same efficiency as man living at sea level (1). Although changes are known to occur in the blood and in the respiratory and cardiovascular systems, modifications at tissue level may be equally important in the process of acclimatization to low-oxygen tensions. Since the respiratory chain of the cell is directly concerned with oxygen utilization, it seemed appropriate to study this multienzyme system by examining the oxidation of reduced pyridine nucleotides in the presence of molecular oxygen. In addition, pyridine nucleotide transhydrogenase, regarded as a key calorigenic and metabolic regulator in biologic oxidations, was also investigated.

2. METHODS

Animals

Adult male guinea pigs were readily obtained from coastal areas for use in the sea-level series and from the central chain of the Andes, at altitudes of about 14,900 feet, for use in the high-altitude series. The isolation of these two places guarantees the purity of the series. Some of the animals used at sea level were born and raised at the laboratory. Both groups were maintained on the same dietary regimen for at least 15 days before experimentation. Temperature and humidity were controlled in the high-altitude series to reproduce sea-level conditions.

Preparation of tissues

The animals were killed by a blow on the head and were bled after decapitation. The required tissues were removed and placed in cold isotonic sucrose. They were weighed on a Roller-Smith balance and then minced in the interior of a glass Potter-Elvehjem homogenizer with thin stainless steel scissors. Nine volumes of cold isotonic sucrose were then added to prepare a 10% homogenate. The total homogenizing time was 1 minute for liver and 1½ minutes for heart and muscle. The procedure was carried out by periods of 30 seconds, standing at 0° C. at least 1 minute.

Reaction mixtures

The standard reaction medium had the following final concentrations in a total volume of 3.0 ml.: 0.04 M nicotinamide; 0.033 M potassium phosphate buffer, pH 7.4; 6.67×10^{-6} M cytochrome C; and 1.67×10^{-4} M of reduced forms of diphosphopyridine and triphosphopyridine nucleotides (DPNH and TPNH), oxidized diphosphopyridine nucleotide (DPN), or combinations thereof. The effect of small changes in hydrogen ion concentration and cytochrome C concentration is shown in figures 1 and 2. Nicotinamide was used to prevent enzymatic inactivation of pyridine nucleotides by specific nucleosidases (2). In general, tissues were used directly as a 10% homogenate except for heart DPNH-oxidase in which case a further dilution to a 1% homogenate was made in cold isotonic sucrose. An initial measurable zero order reaction was obtained by experimentally establishing the optimum amounts of fresh, whole tissues as follows: for DPNH-oxidase system, 5 mg. of muscle and liver and 1 mg. of heart; for TPNH-oxidase system, 30 mg. of muscle and heart and 20 mg. of liver; and for pyridine nucleotide transhydrogenase, 30 mg. of muscle, 20 mg. of liver, and 10 mg. of heart.

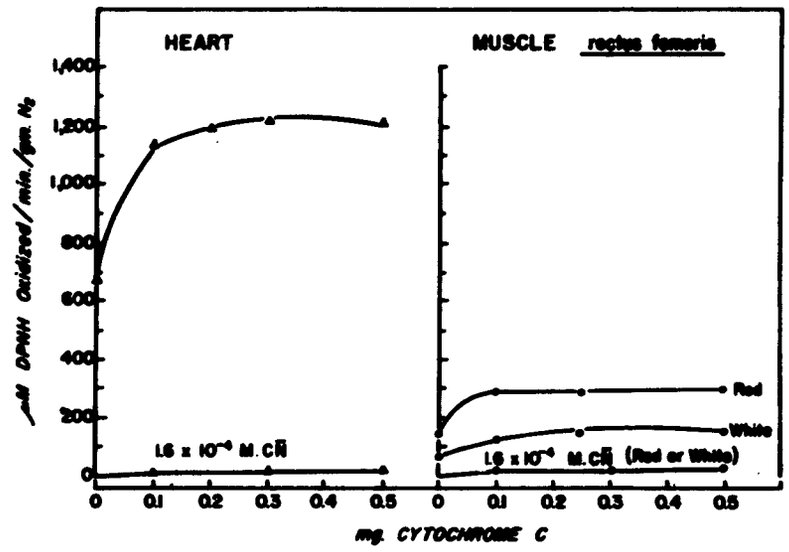

FIGURE 1

DPNH-oxidase activity in heart and muscle rectus femoris (red and white portions) at different levels of cytochrome C.

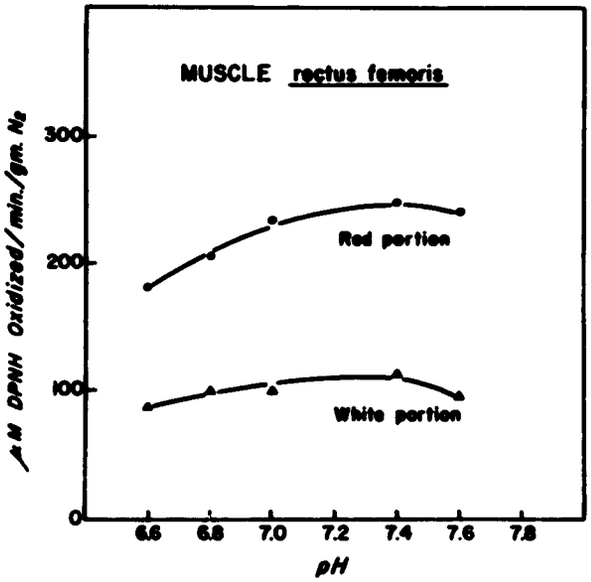

FIGURE 2

Influence of pH on the DPNH-oxidase activity of red and white portions of muscle rectus femoris.

Assay procedure

All the experiments were performed in a model D.U. Beckman spectrophotometer. The oxidation of the reduced pyridine nucleotide was followed at 340 $m\mu$ at intervals of 30 to 60 seconds according to the speed of the reaction. (The experiments were run at room temperature, 28° ± 1.0° C.). Activity was expressed as the number of micromoles of pyridine nucleotide oxidized per minute per gram of total nitrogen. The molar absorbancy of the nucleotides was taken as 6.22×10^3 at 340 $m\mu$. Nitrogen was determined by the Kjeldahl procedure, and the results (table I) are given as milligrams of total nitrogen per gram of wet weight of the tissue.

Source of materials

DPN, DPNH, TPNH, and horse cytochrome C (products of the Sigma Chemical Company) were used in this study. Other commercial products of reagent grade were used, such as nicotinamide, potassium cyanide, and sucrose.

3. RESULTS

DPNH-oxidase system

The results of experiments carried out with three kinds of tissues in guinea pigs from sea level and from altitude are shown in table II, A.

TABLE I

Total nitrogen determination in heart, liver, and muscle (rectus femoris) of guinea pigs from sea level and high altitudes*

Tissue	Sea level	High altitude	P
Heart	25.9 ± 0.5 (12)	25.7 ± 0.2 (12)	NS
Liver	33.9 ± 1.0 (12)	26.3 ± 1.2 (12)	<.01
Muscle			
Whole	30.6 ± 0.6 (12)	29.8 ± 0.5 (5)	NS
Red portion	30.6 ± 0.8 (5)	30.0 ± 0.4 (10)	NS
White portion	29.8 ± 0.2 (5)	29.7 ± 0.4 (10)	NS

Values are means ± S. E. Numbers in parentheses indicate number of animals. Probability values are on t-test significance of difference between means.

*Milligrams of nitrogen per gram of fresh tissue.

TABLE II

Effect of altitude on the specific activity of reduced pyridine nucleotide oxidases and transhydrogenase on guinea pig tissues

Tissue	Sea level	High altitude	P
	A. DPNH-oxidase system		
Heart	983.8 ± 15.4 (11)	1162.1 ± 5.3 (11)	<.01
Liver	226.7 ± 10.4 (11)	269.1 ± 10.8 (11)	<.02
Muscle			
Whole	164.2 ± 5.9 (10)	215.5 ± 8.2 (5)	<.01
Red portion	279.0 ± 6.0 (5)	277.7 ± 5.8 (10)	NS
White portion	134.0 ± 6.6 (5)	135.0 ± 6.4 (10)	NS
	B. TPNH-oxidase system		
Heart	9.6 ± 0.6 (10)	16.4 ± 0.6 (10)	<.01
Liver	39.6 ± 2.0 (10)	43.0 ± 5.5 (9)	NS
Muscle			
Whole	5.2 ± 0.7 (10)	5.0 ± 0.5 (9)	NS
Red portion	5.2 ± 0.5 (4)	5.9 ± 0.6 (9)	NS
White portion	5.0 ± 0.4 (4)	4.8 ± 0.5 (9)	NS
	C. Transhydrogenase		
Heart	39.7 ± 3.7 (10)	64.2 ± 3.2 (10)	<.01
Liver	43.8 ± 4.3 (8)	48.0 ± 3.5 (8)	NS
Muscle			
Whole	13.3 ± 0.6 (8)	17.3 ± 1.0 (8)	<.01
Red portion	19.9 ± 0.9 (5)	21.3 ± 1.4 (8)	NS
White portion	11.5 ± 1.0 (5)	11.8 ± 0.8 (8)	NS

Values are means ± S. E. Numbers in parentheses indicate number of animals. Probability values are based on t-test significance between means.

In the heart, from the latter group of animals, the activity of the DPNH-oxidase system is increased 18.19% over the sea-level group. By analyzing this system in a homogenate of the whole rectus femoris muscle, an increment of 31.2% was found in the animals at altitude. Nevertheless, if the muscle is carefully dissected into its red and white portions and if enzyme activity is tested separately in each one, no difference is found between the two series of animals. That is, the red portion of the rectus femoris muscle from the altitude guinea pig had the same activity as the red portion of the muscle from the sea-level animal. The same is true of the white portion. Regardless of the origin of the animal, the pigmented portion is about twice as active as the white one. As compared with the sea-level group, the apparently higher values of enzyme activity observed in the whole muscle of the altitude animal could be accounted for by the larger ratio of red to white portions found in the muscle (3).

By examining the multienzyme system in liver, a statistically significant difference was found between the two groups of animals, provided the activity was expressed per gram of nitrogen (table II, A). However, if the activity is stated on a fresh-weight basis, the difference is not significant. This suggests that some kind of dilution effect makes the nitrogen content in the altitude liver lower than in the sea-level tissue (table I). The reason for this difference remains to be investigated.

TPNH-oxidase system

To assay this system it is advisable, as has been previously pointed out (4), to deplete the tissue of its complement of pyridine nucleotides. Otherwise, the more active transhydrogenase, in the presence of catalytic amounts of endogenous DPN, could compete for the externally added TPNH, oxidizing it through the DPNH-oxidase system, and leaving no opportunity for the TPNH-oxidase to appear in the experiment as an independent reaction. Since these experiments were carried out in whole homogenates, however, the procedures for depletion usually used in the study of cell fractions were not applicable. Therefore, we were compelled to use the fresh homogenate prepared as described earlier.

The results show that the heart tissue is the only tissue which exhibits a higher TPNH-oxidase activity in the altitude series. The question remains, however, as to whether this increment is a real one or is due to a contamination with transhydrogenase, greatly increased in this tissue (table II, C). A further investigation using cell fractions is imperative. Liver and muscle, on the other hand, showed no difference for the two series of animals (table II, B). Furthermore, the red portion of the muscle had the same activity as the white portion, differing in this respect from the DPNH system which is much more active in the pigmented portion. It should be noted that in liver no difference was found between the activities of the TPNH system and that of the transhydrogenase (table II, B and C). It might be that liver has an excess of DPN so that when TPNH is added it is oxidized mainly through the DPNH-oxidase system because of the transhydrogenase. Consequently, no true conclusions can be drawn as to the activity of the TPNH-oxidase system in this tissue.

Pyridine nucleotide transhydrogenase

The activity of the enzyme was determined by following the rate of oxidation of TPNH in a Beckman cell containing, besides tissue and the standard reaction medium, 0.5 μM. of exogenous DPN. The results obtained in the tissues of both sea-level and altitude animals are shown in table II, C. Since TPNH-oxidase system could not be excluded from the enzymatic assay, the values given for transhydrogenase are, in general, somewhat higher than those which would be expected if the pure enzyme had been used. However, they resemble the real ones much more closely than the figures given for the TPNH system, inasmuch as the latter is less than the transhydrogenase. By comparing both groups of animals, it may be seen that the altitude specimen has 62.0% higher activity in heart and 30.2% higher activity in muscle than the

sea-level specimen. As for liver, it may be concluded that no change in the activity of the enzyme is produced by high-altitude environments. It should be emphasized that the 30.2% increase in the muscle of the altitude group is due solely to transhydrogenase activity, since the TPNH system remains unchanged. Furthermore, the transhydrogenase, like the DPNH-oxidase system, has a higher activity in the red portion of the muscle, which is 81.0% more active than the white portion.

4. DISCUSSION

The purpose of this study was to investigate the effect of chronic hypoxia on the hydrogen transport system of the cell in guinea pigs born at high altitude and kept in the laboratory under conditions of temperature, humidity, and diet similar to those for a control sea-level group. The results, however, must not be attributed exclusively to a low-oxygen pressure, but rather to the combination of factors inherent in the high-altitude environment.

The results of this study show that the tissues from the high-altitude guinea pig tend to have a greater oxidative capacity, as far as the systems under study are concerned, than those from the sea-level specimens. However, the magnitude and type of increment are characteristic of every tissue. The total DPNH-oxidase and transhydrogenase activity is enhanced in heart not only by virtue of an increased specific activity but also because the heart is larger in the altitude guinea pigs (3). The TPNH-oxidase system is also increased in this tissue, but a possible contamination with transhydrogenase would have to be investigated.

In muscle, the increment in the activity of the DPNH-oxidase system is closely linked with the red character of the tissue. Consequently, the enhanced total activity of the whole muscle is due merely to a higher proportion of the pigmented region. In view of these findings, it would be desirable to establish a correlation between the oxidative activity and the pigments, or factors responsible for this activity, as has been attempted previously by Lawry (5, 6) and more recently by Tappan and Reynafarje (3). The increment in the activity of the enzyme transhydrogenase in muscle follows the same pattern of variation as the DPNH-oxidase system. On the other hand, the activity of the TPNH-oxidase system is not significantly changed. Moreover, the red and white portions have the same activity, indicating a lack of correlation between pigmentation and TPNH-oxidative capacity. Therefore, it is unlikely that this system is involved in adaptation to high altitude.

In liver, neither the TPNH-oxidase nor the transhydrogenase is appreciably changed in their activity. The DPNH-oxidase system, nevertheless, appears to be increased at altitude, provided the activity is expressed per gram of nitrogen. If it is expressed per gram of fresh weight, however, no significant difference is found with respect to the sea-level control. Whether a diluting effect is operative in the liver of the high-altitude guinea pig must be elucidated in a further investigation. From these findings it may appear that liver is not affected in its hydrogen transport system as is heart or skeletal muscle. The special physiology of liver, involved in a number of important synthetic reactions using TPNH as a reducing agent, might be the reason for this behavior. If the formation of high-energy phosphate bonds is associated mainly with the oxidation of DPNH (7), and if the reduced coenzyme in the altitude organism is not only produced at a higher rate but is also more efficiently oxidized, it would not be unreasonable to think that this might be one of the mechanisms by which man adapted to high altitude performs physical work with equal efficiency and longer endurance, and with less lactic acid production than at sea level (1).

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